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Molecular cloning and sequence determination of the peplomer protein gene of feline infectious peritonitis virus type I

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Summary. cDNA clones spanning the entire region of the peplomer (S) gene of feline infectious peritonitis virus (FIPV) type I strain KU-2 were obtained and their complete nucleotide sequences were determined. A long open reading frame (ORF) encoding 1464 amino acid residues was found in the gene, which was 12 residues longer than the ORF of the FIPV type II strain 79–1146. The sequences of FIPV type I and mainly FIPV type II were compared. The homologies at the N- (amino acid residues 1–693) and C- (residues 694–1464) terminal halves were 29.8 and 60.7%, respectively. This was much lower than that between FIPV type II and other antigenically related coronaviruses, such as transmissible gastroenteritis virus of swine and canine coronavirus. This supported the serological relatedness of the viruses and confirmed that the peplomer protein of FIPV type I has distinct structural features that differ from those of antigenically related viruses.

Introduction

Feline infectious peritonitis (FIP) is a virus-induced chronically progressive and usually fatal disease in domestic and wild *Felidae*. The causative agent of this disease is FIP virus (FIPV) which belongs to the family *Coronaviridae*. FIPV forms a related antigenic cluster with feline enteric coronavirus, transmissible gastroenteritis virus (TGEV) of swine, canine coronavirus (CCV), and porcine respiratory coronavirus (PRCV) [12, 18, 23]. FIPV has two serological subtypes, type I and type II, which cause similar diseases in animals [8, 9, 20]. When we surveyed antibody positive cats in Japan, about 70 and 30% with FIP were infected with types I and II, respectively [10].

FIPV is an enveloped RNA virus with a single-stranded positive-sense RNA genome. The FIPV virions consist of three main structural proteins, peplomer (S) protein, membrane (M) protein and nucleocapsid (N) protein. A comparison of the antigenicity of M and N proteins of FIPV types I and II revealed that

they are serologically closely related. However, their peplomer proteins were not homologous [5, 8, 9]. The virus genome is about 20 kilobases (kb) long and three major structural protein genes are located in the 3′ half of the genome [3]. The complete nucleotide sequences of these genes have been established with FIPV type II strain 79–1146 [2, 4, 27]. Several other serologically related coronaviruses have also been investigated molecularly, such as TGEV [13, 22], and CCV [11, 28].

To date, immunization against FIP has not been available because of the antibody-dependent enhancement (ADE) phenomenon due to humoral antibody [7, 15, 17, 19, 30]. To clarify the involvement of virion antigens in this phenomenon, Vennema and his collaborators cloned cDNAs for the S, M and N protein genes of FIPV and constructed recombinant vaccinia viruses, each of which contained one of these cDNAs. These recombinant viruses were used for immunization and subsequent virus challenge of kittens. Those authors indicated that only the peplomer protein might be responsible for this phenomenon [26, 27]. FIPV type II has been preferentially used for experimental materials, probably because the proliferative efficiency of FIPV type II in cultured cells is much higher than that of type I. Thus, type I has been little investigated, especially at the molecular biological level. Therefore, it seems important to study FIPV type I, which is more prevalent in Japan than type II. In this study cDNA clones and sequences of the peplomer gene of FIPV type I were compared with those of related viruses. These studies confirmed the serological relatedness between FIPV type I and other coronaviruses.

Materials and methods

Virus and its genomic RNA

FIPV type I, strain KU-2, isolated by Hohdatsu et al. [9], was propagated in *Felis catus* whole fetus cells (fcwf 4). Virus-infected cells were homogenized with a Dounce type homogenizer and cell nuclei were removed by low speed centrifugation. Virions in the supernatants were pelleted through layers of 35% sucrose by centrifugation at 200 000 × **g** for 2 h. Virus genomic RNA was extracted from the virus pellets with SDS-phenol and precipitated with ethanol.

DNA primers

DNA primers were custom-synthesized by Bex Corp. (Tokyo) by using di-amidite chemistry and an automatic DNA synthesizer. Two minus-sense primers were prepared for each cloning study, one of which, located downstream, was used for reverse transcription, and the other, located upstream, for subsequent PCR. The nucleotide sequences and positions of the primers are shown in Table 1. Primer IIMPr-1 and IIMPr-2 were created with reference to the nucleotide sequence [27] of the M protein gene of FIPV strain 79–1146.

cDNA cloning

Genomic template RNA of FIPV type I strain KU2 was reversely transcribed with a negative-sense downstream DNA primer. The template RNA was digested with 0.2 M NaOH at 55 °C for 1 h and a poly dA tail was added to the single-stranded cDNA by

Table 1. Nucleotide sequences of DNA primers

IIMPr-1[a]	5′-TGGGGATCCGATTTTACGTAGTAAGCCCA-3′	(position 745)[e]
IIMPr-2[b]	5′-CGAAGAATTCATATCTGGAAACTTGGTACTC-3′	(position 367)[e]
ISPr-1[a]	5′-GACTCTTCAATATCCAGCTGAA-3′	
ISPr-2[b]	5′-GGGGAATTCAGAGGTAAATAATACTTTAAGTG-3′	
ISPr-3[a]	5′-TGAATCTTTCAACAGTTGCA-3′	(position 2892)[f]
ISPr-4[b]	5′-TTTTGAATTGAATCTGAATGTATTCAGC-3′	(position 2689)[f]
ISPr-5[a,c]	5′-GGGGAATTCGCAGTCATAATTTTGTAGCTC-3′	(position 1924)[f]
ISPr-6[b]	5′-GGGGAATTCTATTGAGGGGCGAGTCAC-3′	(position 1565)[f]
ISPr-7[b]	5-GGAGGATCCTCAGACTTGGCAACGCTAAG-3′	(position 94)[f]
$BEP(dT)_{21d}$	5-CTGTGAATTCTGCAGGATCCTTTTTTTTTTTTTTTTTTTT-3′	

[a]Used as a primer for RT
[b]Used as a primer for PCR
[c]Used as a primer in sequencing
[d]Used as a plus-sence primer for PCR
[e]The position of the bond in FIPV strain 79–1146 M protein gene (Accession No. X56496)
[f]The position of the bond in FIPV strain KU-2 peplomer protein gene

using terminal deoxynucleotidyl transferase. The poly dA-tailed cDNA was amplified by PCR, primed with another 5′ upstream primer and the oligo dT21 primer, which have recognition sites for restriction enzymes BamHI, EcoRI and PstI. DNA fragments larger than 1-kb pairs were obtained by electrophoresis with low melting point agarose, digested with restriction enzymes and cloned into the same restriction sites of pUC18. The cDNA clones were selected by sequencing the nucleotides of the 3′ end that contained the primer sequence.

DNA sequencing and analysis

The cloned cDNA was subcloned into M13mp18/19 and single-stranded DNA was sequenced. The restriction sites used to obtain the cDNA fragments are shown in Fig. 1. The DNA was sequenced by means of dideoxynucleotide chain termination using the Dye Primer cycle sequencing kit (Applied Biosystems). The sequence was resolved with an automated DNA sequencer (Applied Biosystems model 373A).

The sequences determined were then analyzed with the GENETYX computer program (Software Development Co., Ltd.). Homology including the deleted sequence was calculated. The nucleotide sequence data reported in this paper will appear in the GSDB, DDBJ, EMBL and NCBI nucleotide sequence databases with the accession number D32044.

Results

Strategy for cDNA cloning

Since FIPV type I proliferates more slowly than type II, it was not easy to obtain a large quantity of purified viruses. We used PCR to generate sufficient cDNA from a viral template. When the first cDNA was synthesized, the minus-strand DNA primers for the 3′ end necessary for reverse transcription (IIMPr-1) and PCR (IIMPr-2) were prepared on the basis of the nucleotide sequence of the FIPV strain 79–1146 M gene. A poly dA tail was added to the single-strand cDNA by using terminal deoxynucleotidyl transferase, and oligo dT21 was used as the plus-strand primer for PCR. The amplified cDNA was molecularly cloned into the multiple cloning sites of pUC18 and nucleotide sequences at both ends of the clones were determined. Those clones containing the nucleotide sequence of the primer at the 3′ end were selected and those containing it at the 5′ end were referred to prepare DNA primers for the next cDNA cloning. The cDNA cloning experiments were hence performed sequentially. Finally, five cDNA clones (pFPSI-1, 2, 3, 4 and 5), four of which covered the entire peplomer gene, were isolated as shown in Fig. 1. The complete nucleotide sequence of the peplomer gene of FIPV type I was determined by sequencing these cDNA clones. At least five clones for one type of cDNA were sequenced to avoid artifact mutations due to misreading by reverse transcriptase and Taq polymerase for PCR.

The peplomer gene of FIPV type I

A long open reading frame (ORF) was found extending from the second to the fifth cDNA, which was considered to be the peplomer protein of FIPV type I. This ORF is 4392 bases (1464 amino acid residues) long, which is equivalent

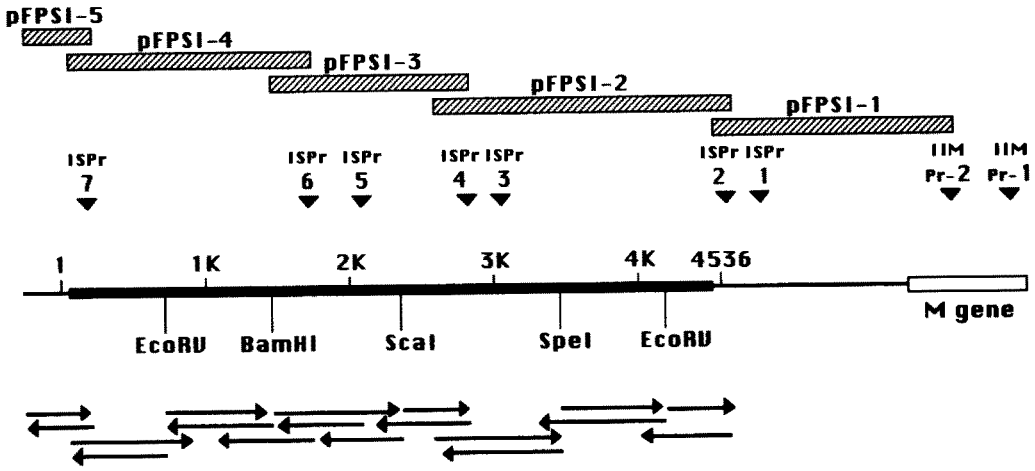

Fig. 1. Physical mapping of cDNA clones obtained and strategies for sequencing them. The solid line represents the peplomer gene. Restriction sites available for subcloning are indicated. cDNA clones are mapped by hatched boxes. Arrows indicate sequenced segments of cDNA clones. Arrowheads indicate the binding locations of DNA primers which are shown in Table 1

to a predicted protein of 163.5 kDa. This gene is 36 bases longer (12 amino acids) than that of FIPV type II reported by De Groot et al. [2]. The total nucleotide and deduced amino acid sequences in the ORF of the peplomer gene of FIPV type I are shown in Fig. 2.

The peplomer protein of FIPV type I also has two hydrophobic segments characteristic of a type I membrane protein with an N-terminal signal sequence (residues 1–28) [29, 31] and a transmembrane domain (residues 1406–1426). The amino acid sequence homology of the latter is completely conserved but that of the former is not. Downstream of the transmembrane domain, there are many cysteine residues. A similar N-terminal signal sequence, C-terminal transmembrane domain and cysteine cluster are present in the peplomer proteins of FIPV type II, CCV and TGEV.

Forty-one potential N-glycosylation sites (NXS,T) are present in the overall peplomer protein, which is six sites fewer than in type II. Their locations are in good accordance at the C-terminal part but less so at the N-terminal part. The peplomer proteins of FIPV type II and TGEV are not cleaved by host cell proteinases [25]. In the peplomer protein of FIPV type I, no sequence was identified as the cleavage motif RRFRR for avian infectious bronchitis virus [1] except for the tetranucleotide sequence RRSR (residues 787–790) as a vestigial cleavage site.

A comparison between FIPV type I and II sequences

The 5′ and 3′ non-coding regions (nucleotides) and the ORFs (amino acids) of the FIPV types I and II peplomer protein gene are aligned in Fig. 3. The 5′ non-coding region is well conserved, but the 3′ non-coding region is not, with

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GAAAGGTAAAATACTCATTAGAAATAATGGTAAGTTACTAAACTTTGGTAATCACTTTGTTAACACACCATGATATTCATAATACTTACACTCCTTAGCG  100
                    **********                                  M  I  F  I  I  L  T  L  L  S  V   11

TTGCCAAGTCTGAAGACGCTCCTCATGGTGTCACCTTACCCCAATTTAATACGTCCCATAACAATGAAAGGTTTGAACTTAATTTCTACAATTTCTTACA  200
  A  K  S  E  D  A  P  H  G  V  T  L  P  Q  F [N  T  S] H  N  N  E  R  F  E  L  N  F  Y  N  F  L  Q   44

AACTTGGGATATACCACCAAACACAGAAACCATTCTAGGAGGTTATCTGCCTTATTGTGGAGCAGGGGTTAATTGTGGGTGGTATAATTTTAGTCAAAGT  300
 T  W  D  I  P  P  N  T  E  T  I  L  G  G  Y  L  P  Y  C  G  A  G  V  N  C  G  W  Y [N  F  S] Q  S   77

GTGGGACAAAATGGTAAGTATGCCTACATAAACACGCAAAATCTGAATATACCGAACGTTCATGGCGTCTATTTTGACGTAAGAGAACATAATAATGACG  400
V  G  Q  N  G  K  Y  A  Y  I  N  T  Q  N  L  N  I  P  N  V  H  G  V  Y  F  D  V  R  E  H  N  N  D  G  111

GTGAGTGGGATGATCGTGATAAAGTTGGCCTATTGATTGCTATACATGGCAACTCGAAGTATAGTTTGCTTATGGTTTTGCAGGATGCTGTGGAAGCTAA  500
  E  W  D  D  R  D  K  V  G  L  L  I  A  I  H  G  N  S  K  Y  S  L  L  M  V  L  Q  D  A  V  E  A  N-  144

TCAGCCCCATGTTGCTGTTAAAATTTGCCATTGGAAGCCAGGTAACATAAGTTCTTATCACGCGTTTAGTGTAAATCTAGGAGATGGTGGTCAATGCGTG  600
 Q  P  H  V  A  V  K  I  C  H  W  K  P  G [N  I  S] S  Y  H  A  F  S  V  N  L  G  D  G  G  Q  C  V   177

TTTAATCAGAGATTTTCATTGGACACCGTATTGACAACTAATGACTTCTATGGCTTCCAGTGGACTGACACCTATGTTGATATCTATCTAGGTGGCACTA  700
F  N  Q  R  F  S  L  D  T  V  L  T  T  N  D  F  Y  G  F  Q  W  T  D  T  Y  V  D  I  Y  L  G  G  T  I  211

TTACTAAAGTGTGGGTTGACAATGATTGGAGCATTGTTGAAGCTAGCATCTCCTATCATTGGAATCGGATTAACTATGGATATTACATGCAATTTGTTAA  800
  T  K  V  W  V  D  N  D  W  S  I  V  E  A  S  I  S  Y  H  W  N  R  I  N  Y  G  Y  Y  M  Q  F  V [N]  244

TCGCACCACTTATTATGCGTATAATAATACTGGTGGTGCAAATTACACACAATTGCAGTTAAGCGAATGCCATACTGATTATTGTGCTGGCTATGCTAAG  900
[R  T] T  Y  Y  A  Y [N  N  T] G  G  A [N  Y  T] Q  L  Q  L  S  E  C  H  T  D  Y  C  A  G  Y  A  K   277

AATGTCTTTGTGCCTATAGATGGTAAAATACCAGAAGACTTCTCCTTTAGTAACTGGTTTTTATTGTCAGATAAATCCACTTTGGTACAAGGTCGTGTTC 1000
N  V  F  V  P  I  D  G  K  I  P  E  D  F  S  F  S  N  W  F  L  L  S  D  K  S  T  L  V  Q  G  R  V  L  311

TTAGTAGTCAACCAGTTTTTGTACAATGCCTTAGGCCTGTACCATCGTGGTCTAACAATACGGCTGTGGTGCATTTTAAAAATGATGCCTTCTGCCCTAA 1100
  S  S  Q  P  V  F  V  Q  C  L  R  P  V  P  S  W  S [N  N  T] A  V  V  H  F  K  N  D  A  F  C  P [N]  344

CGTCACGGCAGATGTTTTGAGGTTCAATCTAAATTTTAGTGACACTGATGTCTATACAGATTCAACTAATGATGAACAGTTGTTTTTTACATTTGAAGAT 1200
[V  T] A  D  V  L  R  F  N  L [N  F  S] D  T  D  V  Y  T  D  S  T  N  D  E  Q  L  F  F  T  F  E  D   377

AATACAACTGCCTCCATAGCCTGTTATAGCAGTGCCAATGTCACTGATTTTCAGCCTGCAAATAATAGTGTCTCTCACATTCCATTTGGCAAAACTGCGC 1300
[N  T  T] A  S  I  A  C  Y  S  S  A [N  V  T] D  F  Q  P  A [N  N  S] V  S  H  I  P  F  G  K  T  A  H  411

ATTTCTGTTTTGCCAATTTTTCTCATTCAATTGTGAGCAGACAGTTTTTGGGCATACTTCCACCAACTGTTCGAGAGTTTGCATTTGGCAGAGATGGATC 1400
  F  C  F  A [N  F  S] H  S  I  V  S  R  Q  F  L  G  I  L  P  P  T  V  R  E  F  A  F  G  R  D  G  S   444

CATTTTTGTCAATGGCTATAAATATTTTAGTTTACCAGCTATCAGGAGTGTTAACTTCTCCATCAGTTCAGTAGAGGAGTATGGCTTTTGGACCATAGCC 1500
 I  F  V  N  G  Y  K  Y  F  S  L  P  A  I  R  S  V [N  F  S] I  S  S  V  E  E  Y  G  F  W  T  I  A   477

TATACTAACTATACAGATGTAATGGTGGATGTTAATGGCACAGCTATTACTAGGCTATTCTATTGTGACTCGCCCCTCAATAGAATTAAGTGTCAACAAT 1600
Y  T [N  Y  T] D  V  M  V  D  V [N  G  T] A  I  T  R  L  F  Y  C  D  S  P  L  N  R  I  K  C  Q  Q  L  511

TAAAGCATGAATTGCCAGACGGGTTTTATTCTGCTAGTATGCTTGTTAAAAAGGATTTACCCAAAACATTTGTTACCATGCCACAGTTTTACCACTGGAT 1700
  K  H  E  L  P  D  G  F  Y  S  A  S  M  L  V  K  K  D  L  P  K  T  F  V  T  M  P  Q  F  Y  H  W  M   544

GAATGTCACGTTACATGTTGTATTAAATGACACAGAGAAAAAGTATGACATCATTCTCGCTAAAGCCCCTGAGCTAGCAGCACTCGCGGATGTACATTTT 1800
[N  V  T] L  H  V  V  L [N  D  T] E  K  K  Y  D  I  I  L  A  K  A  P  E  L  A  A  L  A  D  V  H  F   577

GAAATAGCTCAGGCTAACGGCAGTGTAACTAATGTTACTAGCCTATGTGTCCAAGCAAGACAGTTGGCTCTATTTTATAAGTATACTAGCTTACAAGGTT 1900
E  I  A  Q  A [N  G  S] V  T [N  V  T] S  L  C  V  Q  A  R  Q  L  A  L  F  Y  K  Y  T  S  L  Q  G  L  611

TGTATACTTATTCTAACTTAGTGGAGCTACAAAATTATGACTGCCCCTTCTCACCGCAGCAGTTTAATAATTATTTGCAGTTTGAAACTTTATGTTTTGA 2000
  Y  T  Y  S  N  L  V  E  L  Q  N  Y  D  C  P  F  S  P  Q  Q  F  N  N  Y  L  Q  F  E  T  L  C  F  D   644

TGTGAACCCTGCTGTGGCAGGTTGTAAGTGGTCGTTAGTTCATGATGTCCAGTGGCGTACGCAGTTCGCCACCATTACGGTTTCTTATAAACATGGTTCT 2100
 V  N  P  A  V  A  G  C  K  W  S  L  V  H  D  V  Q  W  R  T  Q  F  A  T  I  T  V  S  Y  K  H  G  S   677

ATGATCACTACCCATGCCAAGGGGCACAGTTGGGGTTTTCAAGATACCTCTGTTTTGGTAAAAGATGAATGTACTGACTACAATATATATGGCTTTCAGG 2200
M  I  T  T  H  A  K  G  H  S  W  G  F  Q  D  T  S  V  L  V  K  D  E  C  T  D  Y  N  I  Y  G  F  Q  G  711

GCACAGGCATTATTAGAAACACCACCTCAAGGTTAGTGGCTGGTCTTTACTACACATCTATTAGTGGTGACCTTCTAGCCTTTAAAAATAGTACTACTGG 2300
  T  G  I  I  R [N  T  T] S  R  L  V  A  G  L  Y  Y  T  S  I  S  G  D  L  L  A  F  K [N  S  T] T  G   744

TGAGATTTTCACTGTAGTGCCATGTGATCTAACAGCACAAGTAGCTGTGATTAATGATGAGATAGTGGGAGCTATAACAGCCGTTAATCAAACTGATCTG 2400
 E  I  F  T  V  V  P  C  D  L  T  A  Q  V  A  V  I  N  D  E  I  V  G  A  I  T  A  V [N  Q  T] D  L   777

TTTGAGTTCGTAAATAACACACAGGCGAGAAGATCACGTAGTTCAACACCAAATTTTGTAACATCCTATACTATGCCACAATTTTATTACATAACAAAAT 2500
F  E  F  V [N  N  T] Q  A  R  R  S  R  S  S  T  P  N  F  V  T  S  Y  T  M  P  Q  F  Y  Y  I  T  K  W  811

GGAATAATGACACATCGTCCAATTGTACATCTGCCATTACCTACTCCTCTTTTGCTATTTGTAATACTGGTGAGATTAAATATGTTAATGTCACTCATGT 2600
  N [N  D  T] S  S [N  C  T] S  A  I  T  Y  S  S  F  A  I  C  N  T  G  E  I  K  Y  V [N  V  T] H  V   844
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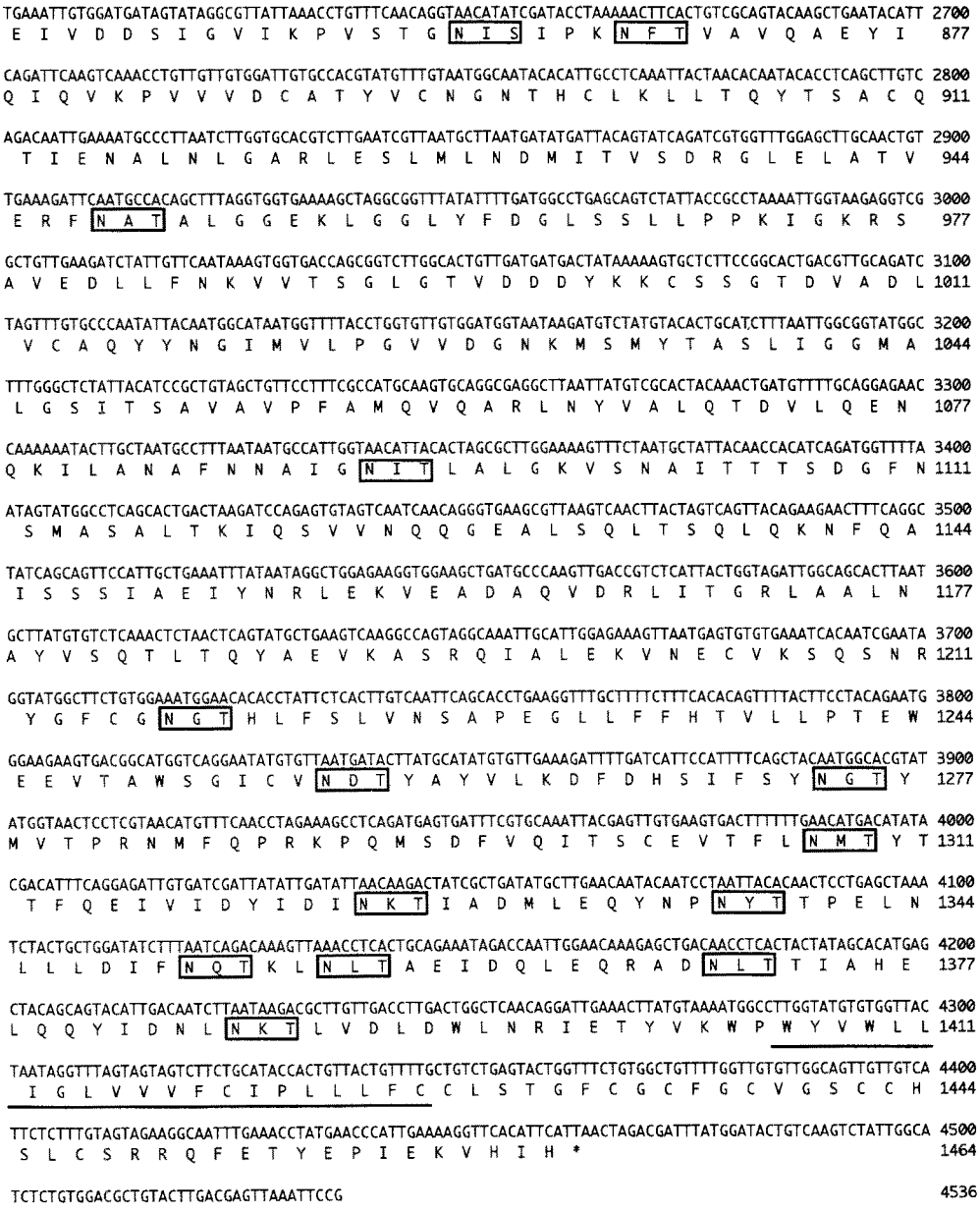

Fig. 2. Nucleotide and deduced amino acid sequences of the peplomer gene of FIPV type I. The putative signal sequence and transmembrane segment are identified by underlines. Potential N-glycosilation sites (NXS, NXT) are indicated by boxes. The arrowhead indicates a presumptive vestigial cleavage site of the peplomer protein to cellular proteinases. The coronavirus-conserved nucleotide sequence is identified by asterisks

sequence homologies of 84.1 and 43.4%, respectively. The consensus sequence ACTAAACTT appearing in most coronaviruses in these regions is conserved only in the 5′, not in the 3′ non-coding region of FIPV type I. The alignment of the ORF for the peplomer protein demonstrates that the degree of sequence

a.

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I:   GAAAGGTAAA ATACTCATTA GAAATAATGG TAAGTTACTA AACTTTGGTA ATCACTTTGT TAACACACC
     *** *****   ********* ********** *** ***** ********** **** ** ** ***    **
II:  GAAGGGTAAG TTACTCATTA GAAATAATGG CAAGCTACTA AACTTTGGTA ATCATTTAGT TAATGTGCC
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b.

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I:   MIFIILTLLS VAKSEDAPHG VTLPQFNTSH NNERFELNFY NFLQTWDIPP NTETILGGYL PYCGAGVNCG WYNFSQSVGQ NGKYAYINTQ NLNIPNVHGV   100
     **     **              *    *          *    *  *            *        *  *  *  *     *  *       *        * *
II:  MIVLVTCLL- ---------- -LLCSYHTVL STTNNECIQV NVTQLAGNEN LIRDFLFSNF KEEGSVVVGG YY--PTEVWY NCSRTARTTA FQYFNNIHAF    86

     YFDVREHNND GEWDDRDKVG LLIAIHGNSK YSLLMVLQDA VEANQPHVAV KICHWKPGNI SSYHAFSVNL GDGGQCVFNQ R---FSLDTV LTTNDFYGFQ   197
     ** * *          * *   **   **            *  *            *  *   *   *  *  *       *     *   **           **
     YF-VMEAMEN STGNARGK-P LLFHVHGEPV SVIISAYRDD VQQRPLLKHG LVCITKNRHI -NYEQFTSN- QWNSTCTGAD RKIPFSVIPT DNGTKIYGLE   183

     WTDTYVDIYL GG-----TIT KVWVDNDWSI VEASISYHWN RINYGYYMQF VNRTTYYAYN NTGGANYTQL QLSECHTDYC AGYAKNVFVP -IDGKIPEDF   291
     * *  *  *   *      *    *  *        * *          *  *  *   ***  * ** *     *      *   *  *** *** *    * **  *
     WNDDFVTAYI SGRSYHLNIN TNWFNNVTLL YSRS-STATW EYSAAYAYQG VSNFTYYKLN NTNGLKTYEL CEDYEH---C TGYATNVFAP TSGGYIPDGF   278

     SFSNWFLLSD KSTLVQGRVL SSQPVFVQCL RPVPSWSNNT AVVHFKNDAF ------CPNV TADVLRFNLN FSDTDVYTDS TNDEQLFFTF EDNTT-ASIA   384
     ** *****    ** * **     **    **  ****          *    *         *  * ** ***** *      *            *   ***   *
     SFNNWFLLTN SSTFVSGRFV TNQPLLINCL WPVPSFGVAA QEFCFEGAQF SQCNGVSLNN TVDVIRFNLN F------TAD VQSGMGATVF SLNTTGGVIL   372

     CYSSANVTDF QPANNSVSHI PFGKTAHFCF ANFSHSIVSR QFLGILPPTV REFAFGRDGS IFVNGYKYFS LPAIRSVNFS ISSVEEYGFW TIAYTNYTDV   484
       *    *        *   * *** *                  ** *** *  * *    *     ***  **    *    *          ** ***** **
     EISCYSDTVS ESSSYSYGEI PFGITDGPRY CYVLYNGTAL KYLGTLPPSV KEIAISKWGH FYINGYNFFS TFPIGCISFN LTTGVSGAFW TIAYTSYTEA   472

     MVDVNGTAIT RLFYCDSPLN RIKCQQLKHE LPDGFY-SAS MLVKKDLPKT FVTMPQF--Y HWMNVT--LH VVLNDTEKKY DIIL--AKAP ELAALADVHF   577
      * *  ***     ** *  *  *** **    *  ***  **   *     *    *  * *  *    * *  *     *          *     *        **
     LVQVENTAIK NVTYCNSHIN NIKCSQLTAN LNNGFYPVAS SEV-GFVNKS VVLLPSFFTY TAVNITIDLG MKLSGYGQPI ASTLSNITLP MQDNNTDV-Y   570

     EIAQANGSVT NVTSLCVQAR QLALFYKYTS LQGLYTYSNL VELQNYDCPF SPQQFNNYLQ FETLCFDVNP AVAGCKWSLV HDVQWRT--- --QFATITVS   672
      *     **   * * *        *           *            *** *    ****  *   *    *   * **      **  **              *
     CIRSNQFSV- YVHSTCKSSL WDNIFNQDCT DVLEAT---- AVIKTGTCPF SFDKLNNYLT FNKFCLSLSP VGANCK---- FDVAARTRTN EQVVRSLYVI   661
                           ▼
     YKHGSMITTH AKGHSWGFQD TSVLVKDECT DYNIYGFQGT GIIRNTTSRL VAGLYYTSIS GDLLAFKNST TGEIFTVVPC DLTAQVAVIN DEIVGAITAV   772
     *  *  *        * *  *  ***  * ** ******  *  **** * * *   ****** * **** ***    * *  * ** *  ** ***    **** *
     YEEGDNIVGV PSDNS-GLHD LSVLHLDSCT DYNIYGRTGV GIIRRTNSTL LSGLYYTSLS GDLLGFKNVS DGVIYSVTPC DVSAQAAVID GAIVGAMTSI   760

     NQTDLFEFVN NTQARRSRSS TPNFVTSYTM PQFYYITKWN NDTSSNCTSA ITYSSFAICN TGEIKYVNVT HVEIVDDSIG VIKPVSTGNI SIPKNFTVAV   872
     *   *       *       *   *    **             **    *    ****    *   *     *** *      * *    * ****   ** ***  *
     NSELLGLTHW TTTPNFYYYS IYN----YTS ERTRGTAIDS ND--VDCEPV ITYSNIGVCK NGALVFINVT H------SDG DVQPISTGNV TIPTNFTISV   848

     QAEYIQIQVK PVVVDCATYV CNGNTHCLKL LTQYTSACQT IENALNLGAR LESLMLNDMI TVSDRGLELA TVERFNAT-- ---------A LGGEKLGGLY   961
     * ** *     **  *** ** ****  * ** **** ***** ** **  *** **      *   **   * **  ** ** *              **  ****
     QVEYMQVYTT PVSIDCARYV CNGNPRCNKL LTQYVSACQT IEQALAMGAR LENMEVDSML FVSENALKLA SVEAFNSTEN LDPIYKEWPS IGGSWLGGL-   948

     FDGLSSLLPP KIGKRSAVED LLFNKVVTSG LGTVDDDYKK CSSGTDVADL VCAQYYNGIM VLPGVVDGNK MSMYTASLIG GMALG-SITS AVAVPFAMQV  1060
      * * *     *    ** ** *** ****** ***** ***  *  * * *** ********** *****    * * ****** * *  **      *** ***  *
     KDILPS-HNS KRKYGSAIED LLFDKVVTSG LGTVDEDYKR CTGGYDIADL VCAQYYNGIM VLPGVANADK MTMYTASLAG GITLGALGGG AVAIPFAVAV  1046

     QARLNYVALQ TDVLQENQKI LANAFNNAIG NITLALGKVS NAITTTSDGF NSMASALTKI QSVVNQQGEA LSQLTSQLQK NFQAISSSIA EIYNRLEKVE  1160
     ********** ****  ** * ****** *** *** * ***   **  ** *     * ** *  * *** ** * ** ** ***  *********   *****
     QARLNYVALQ TDVLNKNQQI LANAFNQAIG NITQAFGKVN DAIHQTSQGL ATVAKALAKV QDVVNTQGQA LSHLTVQLQN NFQAISSSIS DIYNRLDELS  1146

     ADAQVDRLIT GRLAALNAYV SQTLTQYAEV KASRQIALEK VNECVKSQSN RYGFCGNGTH LFSLVNSAPE GLLFFHTVLL PTEWEEVTAW SGICVND---  1257
     ********** *** **** * *****  ***  **** *  * ***** ***  * ******** **** * **  *  ******* **  * **** ****  *
     ADAQVDRLIT GRLTALNAFV SQTLTRQAEV RASRQLAKDK VNECVRSQSQ RFGFCGNGTH LFSLANAAPN GMIFFHTVLL PTAYETVTAW SGICASDGDR  1246

     TYAYVLKDFD HSIF-SYNGT YMVTPRNMFQ PRKPQMSDFV QITSCEVTFL NMTYTTFQEI VIDYIDINKT IADMLEQYNP NYTTPELNLL LDIFNQTKLN  1356
     *   * **      *           *** * * **    **** **  * * *  * *      *   ****** *   * ** * * * * **   *****   **
     TFGLVVKDVQ LTLFRNLDDK FYLTPRTMYQ PRVATSSDFV QIEGCDVLFV NATVIDLPSI IPDYIDINQT VQDILENYRP NWTVPE--FT LDIFNATYLN  1344

     LTAEIDQLEQ RADNLTTIAH ELQQYIDNLN KTLVDLDWLN RIETYVKWPW YVWLLIGLVV VFCIPLLLFC CLSTGFCGCF GCVGSCCHSL CSRRQFETYE  1756
     ** *** **  *   *      **   *** *  *** * *** ********** ********** ********** * *** ***  ** ******  ******* **
     LTGEIDDLEF RSEKLHNTTV ELAILIDNIN NTLVNLEWLN RIETYVKWPW YVWLLIGLVV VFCIPLLLFC CFSTGCCGCI GCLGSCCHSI CSRRQFENYE  1444

     PIEKVHIH                                                                                                    1464
     ****** *
     PIEKVHVH                                                                                                    1452
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c.

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I:   TAAC TAGACGATTT ATGGATACTG --TCAAGTCT ATTGGCATCT --CTGTGGAC GCTGTACTTG ACGAGTTAAA TTCCG
     ***  *  *  * ** * ****  **   * ** **   *  * *     **    *   *  ***    * *  **  * *
II:  TAAA TTTA-AAGTT AAGGATGTTG AATAAATTCC TTAAGAACTA AACTTATTAG TCATTAC--- AGGTCTTGTA TGGAC
```

Fig. 3. Alignments of non-coding nucleotide sequences and amino acid sequence of the peplomer gene between FIPV types I (top) and II (bottom) **a**, **c** Nucleotides sequences of the 5′ (**a**) and 3′ (**c**) non coding region; **b** amino acid sequence of the peplomer protein. Identical sequences are marked with asterisks. Dashes indicate the most probable deletion site at that position. The arrowhead indicates the position at which the amino acid sequence was divided into the N-terminal and C-terminal halves at the assessment of homology

Table 2. Homology of the entire peplomer protein amino acid sequence among FIPV, CCV and TGEV (%)[a]

	FIPV[b] type II	CCV[c]	TGEV[d]
FIPV type I	45.9	45.7	45.1
FIPV type II		90.9	80.9
CCV			79.3

[a]Homology including deleted sequences
[b]FIPV strain 79–1146 (accession number D00150)
[c]CCV strain Insavc-1 (accession number D13096)
[d]TGEV strain Purdue (accession number M21950)

Table 3. Homology at the N-terminal (1–693) amino acid sequence and C-terminal (694–1464) amino acid sequence (%)[a]

	FIPV type I	FIPV type II	CCV	TGEV
FIPV type I		29.8	30.1	29.1
FIPV type II	60.7		85.7	63.4
CCV	60.0	95.5		62.1
TGEV	60.1	96.5	94.6	

[a](Top, right) N-terminal half amino acid sequence homology; (bottom, left) C-terminal half homology

homology is very low in the N-terminal half, but higher in the C-terminal half. The sequence homology data of the peplomer proteins were analyzed and are summarized in Table 2 and Table 3, in which FIPV type I is compared with FIPV type II, TGEV and CCV. Amino acid sequence homology of the entire peplomer protein area was only about 45% between each virus and FIPV type I (Table 2). Table 3 shows homology at the N-terminal (residues 1–693) amino acid sequence and C-terminal (694–1464) amino acid sequence of FIPV type I. While the C-terminal half showed high homology with FIPV type II, CCV and TGEV, FIPV type I showed homology of only about 60% for the other viruses. This table also shows that FIPV type I is distinct from the other three coronaviruses in terms of amino acid sequence homology of the peplomer protein.

Discussion

In this study, we first established cDNA clones and sequenced the nucleotides of the peplomer protein gene of FIPV type I, to compare the sequence of FIPV type I with those of antigenically related viruses in the family *Coronaviridae*.

FIPV forms a related serological cluster with TGEV, CCV and PRCV in the coronavirus family [12, 18, 23]. In this cluster, FIPV type I is considered

to be very discriminative to TGEV, CCV and even FIPV type II, based upon the reactivity with established monoclonal antibodies to the peplomer protein [5, 8, 9]. Jacobs et al. have reported that there is a great divergence (30% homology) between FIPV II and TGEV at the first N-terminal part of the peplomer protein despite a high level of conservation (94%) with only 74 amino acid substitutions at the residual C-terminal part. They stated that this divergence could not be derived from selection of neutralizing antibodies but would be generated by recombination with a related virus [13]. However, a much larger diversity was found in the corresponding region (residues 1–290) between FIPV types I and II (25.4%) and the homology in the residual N-terminal part (residues 291–693) was also very low (32.9%). Furthermore, as shown in Table 2, the amino acid sequence homology was high in the peplomer proteins among FIPV type II, TGEV and CCV. That is, the homology of the entire peplomer protein of FIPV type II with that of CCV is 90.9%, and that between FIPV type II and TGEV is 80.9%. In contrast, the homology between FIPV types I and II is 45.9% over the entire region, with 60.7% in the C-terminal half (residues 694–1464) and only 29.8% in the N-terminal half (residues 1–693). Consequently, the amino acid sequence of the FIPV type II peplomer protein is much more homologous with those of CCV and TGEV than with that of FIPV type I. These results support the serological relatedness by revealing the extensive heterogeneity of the peplomer protein of FIPV type I within the cluster.

We found greater diversity in the corresponding region between FIPV types I and II. Such divergent N-terminal domains are probably involved in the construction of the globular head structures of the coronavirus peplomer protein. These structures must have important roles for virus infection, when viruses attach to cellular receptor sites. It is unlikely that virions of both FIPV types bind to the same receptors on the cellular membrane.

The ADE phenomenon has hindered the control of FIP by immunization with vaccines. In fact, neither inactivated virions nor recombinant antigens in the vaccinia virus vector induced protection in vaccinated animals against subsequent virus challenge [24]. Vennema et al. have postulated that only the peplomer protein is responsible for this phenomenon [26, 27]. This protein is also thought to be the most suitable for use as an FIP vaccine. Whether the epitopes responsible for ADE and protection against viral infection are separable or not will be a fundamental problem in developing usable FIP vaccines. At present, we have no conclusive information about this. Even if a protective vaccine for either type of FIPV is developed in the future, one for the other type will also be required, because the peplomer proteins are quite different. Since FIP is usually a fatal disease and its morbidity rate is not low, effective vaccines are desirable for control of this disease.

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